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Effect of intracardiac repair on biosynthesis of thromboxane A_2 and prostacyclin in children with a left to right shunt

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Abstract

***Objective*—To investigate the effect of intracardiac repair on the abnormal biosynthesis of prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) in children with congenital heart disease and increased pulmonary blood flow.**

***Design*—A prospective study with immunoaffinity chromatography and gas chromatography-mass spectrometry to measure the urinary excretion products of PGI_2 (2,3-dinor-6-oxo-prostaglandin (PG) $F_{1\alpha}$ (2,3-dinor-6-oxo-$PGF_{1\alpha}$)) and TXA_2 (2,3-dinor-TXB_2) before operation, in the first 12–24 h after operation, and at discharge from hospital.**

***Setting*—A supraregional referral centre for patients with congenital heart disease.**

***Patients*—15 patients aged 2 to 60 months (median 7 months) with a left to right shunt who underwent intracardiac repair.**

***Results*—The preoperative 2,3-dinor-TXB_2 excretion rate was greater than that found previously in a control group of 16 healthy children with a median (range) age of 24 (6–36) months (1159(201) *v* 592(122) ng/g creatinine in controls, P = 0·006). The excretion rate rose after operation to 9600(3832) ng/g creatinine (P = 0·01) and decreased before discharge to 1071(191) ng/g creatinine (NS), but remained greater than that of the control group (P = 0·014). Before operation 2,3-dinor-6-oxo-$PGF_{1\alpha}$ excretion rates were similar to those of the healthy children (482(68) *v* 589(95) ng/g creatinine in controls) but increased after operation to 19 668(11 162) ng/creatinine (P = 0·002) and fell at discharge to 1621(245) ng/g creatinine although this was higher than both preoperative and control rates (P = 0·005 and P = 0·0002 respectively). The preoperative ratio of 2,3-dinor-TXB_2 to 2,3-dinor-6-oxo-$PGF_{1\alpha}$ excretion was greater than that of the control group (3·2(0·8) *v* 1·3(0·22) in controls, (P = 0·005)), decreased significantly after operation to 0·9(0·13) (P = 0·016), and changed little, to 0·7(0·12), before discharge. The last two ratios were similar to those in normal children and significantly lower than those before operation (P = 0·004).**

***Conclusion*—In children with a left to right shunt the ratio of the excretion rates of the metabolites of TXA_2 and PGI_2 was abnormal before operation, which favoured vasoconstriction and platelet aggregation, but had decreased at discharge from hospital. The increase in excretion of PGI_2 metabolites over TXA_2 metabolite after intracardiac repair augurs well for pulmonary vascular recovery.**

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The pulmonary circulation is exposed to a high shear stress from birth in children with congenital heart disease and a left to right shunt. Structural abnormalities are known to be present by 2 months of age but endothelial dysfunction precedes morphological damage.[1-3] Recent studies have shown that the metabolism of several vasoactive mediators is abnormal in children with pulmonary hypertensive congenital heart disease.[4,5] An imbalance in eicosanoid biosynthesis with an excess of thromboxane A_2 (TXA_2) over prostacyclin (PGI_2) may occur,[4] as in pulmonary hypertension of other aetiologies.[6] The vasoconstrictor TXA_2 is a proaggregatory substance whereas PGI_2 acts as a physiological antagonist to TXA_2, relaxing vascular smooth muscle and inhibiting platelet aggregation.[7-9] Thus before operation, children with pulmonary hypertensive congenital heart disease have an imbalance in favour of vasoconstriction and platelet aggregation.

Cardiopulmonary bypass damages the pulmonary endothelium in all patients and those with pulmonary hypertension are particularly vulnerable.[10-14] Pulmonary hypertension might be mediated by an increase in a vasoconstrictor or decrease in a vasodilator substance. The circulating concentration of the vasoactive substance derived from the endothelium, endothelin, is high in such children,[5] but the release of nitric oxide in response to acetylcholine may be impaired.[15] We hypothesised that if the abnormal ratio of metabolites of TXA_2: PGI_2 that may be present in children with an increased pulmonary blood flow reverted to normal after surgical closure of the shunt this should reduce the dominant effect of TXA_2 after the operation and might provide evidence for concomitant endothelial cell recovery. Persistent abnormalities of eicosanoid biosynthesis might indicate residual haemodynamic disturbance. Therefore we investigated the biosynthesis of TXA_2 and PGI_2 in a group of children with a left to right shunt and pulmonary hypertension before, during, and after intracardiac repair.

Both PGI_2 and TXA_2 have short half lives in the circulation and hydrolyse spontaneously to 6-oxo-prostaglandin (PG) $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) and TXB_2. Previous investigators have reported transient increases in plasma concentrations of both eicosanoids.[16 17] Blood sampling may cause local endothelial trauma and platelet activation that can result in artifactual increases of both eicosanoids in blood samples.[18–20] A non-invasive assessment of the biosynthesis of PGI_2 and TXA_2 can be made by measuring eicosanoid metabolites in urine. Excretion of 6-oxo-$PGF_{1\alpha}$ and TXB_2 largely reflect renal biosynthesis of eicosanoids[21 22] whereas 2,3-dinor-6-oxo-$PGF_{1\alpha}$ and 2,3-dinor-TXB_2 reflect biosynthesis of PGI_2 and TXA_2 outside the kidney.[23 24] We measured the urinary excretion of these 2,3-dinor metabolites before operation during the first 12–24 hours after the operation, and immediately before discharge from hospital in a group of pulmonary hypertensive children with a left to right shunt. These children were younger than those previously studied, which reflects the trend towards earlier intracardiac repair.[16 17]

Patients and methods

PATIENTS

The 12 hour urinary excretion of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ and 2,3-dinor-TXB_2 was measured in 15 children aged 2—60 (median 7) months (table). Preoperative and discharge urine samples were collected overnight, as were the control samples. Twelve of these children were studied before operation, 12 between 12 and 24 hours after operation, and 11 immediately before discharge at 6–26 (median 12) days after operation. Only six children could be studied serially. No child had renal insufficiency, before or after operation. The parents of all patients gave informed consent.

Before the operation, all the children had a left to right shunt and a chest radiograph showing cardiomegaly and pulmonary plethora. Fourteen children were treated with frusemide and digoxin. Also 10 children received spironolactone and two received captopril. Most of the children underwent intracardiac repair after a cross sectional echocardiographic and Doppler assessment, without preoperative cardiac catheterisation (table). Of these cases, eight had a reliable non-invasive estimation of right ventricular pressure that varied between 45 and 90 (mean 61) mm Hg. The pulmonary arterial pressure was high in all catheterised patients. Estimation of pulmonary artery pressure was made within the week before surgery. Surgery was entirely successful in 12 children but three had a difficult postoperative course. One child had severe pulmonary hypertensive crises and two had residual left to right shunts and remained in heart failure. One of these required reoperation. All 15 patients survived.

METHODS

Analysis of eicosanoid metabolites

Urinary excretion rates of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ and 2,3-dinor-TXB_2 were measured with immunoaffinity chromatography and gas chromatography-mass spectrometry as described previously.[25] Briefly, a well mixed sample of 30–50 ml was stored at –20°C until assayed. Urine samples (10 ml) were diluted 1:1 by volume with buffer at pH 8·0 and internal standards of $^2H_4$ 2,3-dinor-TXB_2 and $^2H_4$ 2,3-dinor-6-oxo-$PGF_{1\alpha}$ (5 ng each) were added. Eicosanoids were extracted with cyanogen bromide activated sepharose columns containing immobilised antibodies that had been raised against 6-oxo-$PGF_{1\alpha}$ and TXB_2 and that cross reacted with their respective 2,3-dinor metabolites. Urine samples were applied under vacuum to the columns, which were washed with water (10 ml). Eicosanoids were eluted by addition of 0·5 ml acetone:water (95:5) and rotation of the columns for 15 min. Samples were taken to dryness (N_2 stream) and were derivatised as 3,5-bis-trifluoromethylbenzyl esters and trimethylsilyl ethers. They were analysed with a VG 70-SEQ gas chromatograph-mass spectrometer in the electron capture mode with

Clinical and eicosanoid data

Patient	Age (months)	Sex	Diagnosis	Preoperative PAp or RVp	Before operation: DN-TXB_2 (ng/creat)	Before operation: DN-6-oxo (ng/creat)	Before operation: Ratio	Intensive care unit 12–24 h after operation: DN-TXB_2 (ng/g creat)	Intensive care unit 12–24 h after operation: DN-6-oxo (ng/g creat)	Intensive care unit 12–24 h after operation: Ratio	Discharge: DN-TXB_2 (ng/g creat)	Discharge: DN-6-oxo (ng/g creat)	Discharge: Ratio
1	2	F	VSD	70	2285	736	3·10	12720	11776	1·08	—	—	—
2	3	F	AVSD Tri	50	931	88	10·58	1326	842	1·58	2247	1968	1·14
3	3	M	AVSD Tri	—	1110	609	1·82	37182	103428	0·36	904	1295	0·70
4	3	F	VSD	60	2606	640	4·07	7814	10593	0·74	1342	2333	0·58
5	4	F	AVSD Tri	50	—	—	—	1743	2019	0·86	690	2661	0·26
6	7	M	VSD	45	1146	219	5·24	2930	3211	0·91	—	—	—
7	14	F	AVSD	49/15	734	605	1·21	—	—	—	—	—	—
8	18	F	VSD	73/18	—	—	—	14705	38052	0·39	1002	711	1·41
9	24	M	AVSD	—	376	515	0·73	7161	6353	1·13	764	1277	0·60
10	30	F	VSD	—	1132	410	2·76	—	—	—	1562	2395	0·65
11	36	M	ASD	—	504	350	1·44	822	738	1·11	826	1209	0·68
12	60	F	VSD	47	398	412	0·97	—	—	—	298	736	0·40
13	4	M	DORV	90	1504	922	1·63	5744	9545	0·60	1807	465	3·89 R
14	4	M	VSD	80	—	—	—	61404	60531	1·01	1124	440	2·56 R
15	10	M	AVSD Tri	74/24	1183	283	4·18	2172	541	4·01	—	—	PHC
Control values					592 (122)	589 (95)	1·3 (0·22)						

AVSD, atrioventricular septal defect; creat, creatinine; DN-TXB_2, 2, 3-dinor-TXB_2; DN-6-oxo, 2, 3-dinor-6-oxo-$PGF_{1\alpha}$, DORV, double outlet right ventricle; PHC, pulmonary hypertensive crises; PAp, pulmonary arterial pressure; PDA, patent ductus arteriosus; R, residual VSD; RVp, right ventricular systolic pressure; Tri, trisomy 21; VSD, ventricular septal defect.

methane as reagent gas. Carboxylate anions at mass:charge (m:z) ratio 557 were monitored for 2,3-dinor-6-oxo-$PGF_{1\alpha}$ and 2,3-dinor-TXB_2 and at m:z 561 for the deuterated internal standards. The detection limit for each eicosanoid was 5 pg/ml. Urinary creatinine concentrations were measured with standard biochemical methods. No patient took aspirin or a related substance and all urine samples were found to be free of salicyluric acid.[26]

ANALYSIS OF DATA

The results were expressed as mean (SEM) ng eicosanoid/g urinary creatinine to allow for changes in renal function and urine volume. All data were transformed logarithmically to obtain normally distributed data before analysis. Urinary eicosanoid excretion rates were compared with those found previously in a control group of 16 normal children aged 6–36 (median 24) months with the unpaired Student's t test.[4] Differences were considered significant at $P < 0.05$. For comparison of preoperative, intensive care unit (ICU), and discharge values, only paired urine samples from the same patients were studied. A one way analysis of variance (ANOVA) for repeated measures was used to detect differences in the excretion rates of eicosanoids before operation, on the intensive care unit, and at discharge. When significant differences were found ($P < 0.05$) the Bonferroni procedure for multiple comparisons was used to test for pairwise differences. These were considered significant at $P < 0.017$. In the three children who had a complicated postoperative course eicosanoid biosynthesis was considered to be different from the group without postoperative complications if their values fell above or below the upper and lower 95% confidence interval (95% CI) of that group.

Results

The mean preoperative excretion rate of 2,3-dinor-TXB_2 was 1159(201) ng/g creatinine, significantly higher than the excretion rate of 592(122) ng/g creatinine in the healthy control group ($P = 0.006$). Excluding the three children who had a complicated postoperative course, the rate of 2,3-dinor-TXB_2 excretion increased from the preoperative rate of 1159(697) to 9600(3832) ng/g creatinine in the intensive care unit ($P = 0.014$), and was 1071(191) ng/g creatinine immediately before discharge $P = 0.035$ (NS). Its rate of excretion on discharge from hospital was not significantly different from the preoperative rate, but was significantly greater than in the control group ($P = 0.014$).

Before operation, the mean urinary excretion rate of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ was 482(68) ng/g creatinine, similar to the control group (589(95) ng/g creatinine). At 12–24 hours after operation, in the intensive care unit, the rate rose to 19 668(11 162) ng/g creatinine ($P = 0.002$), and before discharge, it had fallen to 1621(245) ng/g creatinine. This value was higher than both the preoperative baseline values ($P = 0.005$) and the control group ($P = 0.0002$). The ratio of 2,3-dinor-TXB_2: 2,3-dinor-6-oxo-$PGF_{1\alpha}$ excretion before operation was 3·2(0·8), compared with a control ratio of 1·3(0·22) ($P = 0.005$). At 12–24 hours after operation, the ratio decreased to 0·9(0·13) ($P = 0.016$). This ratio was similar at the time of discharge (0·7(0·12)) and less than the preoperative value ($P = 0.004$). The ratio at discharge was not significantly different from the control value.

Concerning the three children who had a complicated postoperative course, in the two who had a residual left to right shunt, the excretion of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ at the time of discharge was below the lower 95% CI (440 and 465 compared with upper and lower 95% CIs of 1056 and 2185 ng/g creatinine) for the rest of the patients, although excretion of the 2,3-dinor-TXB_2 was similar. The ratios of 2,3-dinor-TXB_2: 2,3-dinor-6-oxo-$PGF_{1\alpha}$ at discharge were above the 95% CI of the other patients (2·6 and 3·9 compared with lower and upper 95% CIs of 0·4–1·0). One of these children had a postoperative peak right ventricular pressure of 50 mm Hg and a pulmonary:systemic flow ratio of 2:1. He subsequently underwent a second and successful intracardiac repair. On that occasion the excretion of the 2,3-dinor-6-oxo-$PGF_{1\alpha}$ at the time of discharge was greater than that seen in any other child and the ratio was 0·5.

The child who had postoperative pulmonary hypertensive crises had a lower excretion rate of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ at 12–24 hours after surgery, during the crises, than any other child in the group. During the time the child was ill in the intensive care unit the eicosanoid metabolite excretion ratio was 4·0, well above the 95% CI (0·6–1·0) for the postoperative group of patients with an uncomplicated course.

Discussion

Before operation, the 12 hour excretion rate of 2,3-dinor-TXB_2 was high whereas that of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ was at the lower limit of the control group. This significantly increased the ratio. These findings are consistent with those in our previous study in which we found high 2,3-dinor-TXB_2 excretion in children with congenital heart disease and a high pulmonary blood flow, compared with the same control group.[4] In the present study, after operation excretion of both eicosanoid metabolites was noticeably high for longer than has previously been reported.[16,17] Other investigators reported an increase in the plasma concentration of 6-oxo-$PGF_{1\alpha}$ and TXB_2 in children during cardiopulmonary bypass that quickly returned to the preoperative concentration in the recovery room.[16,17] In patients undergoing palliative cardiac surgery without cardiopulmonary bypass the plasma concentration of TXB_2 did not change whereas that of 6-oxo-$PGF_{1\alpha}$ increased,[17] possibly attributable to intravascular manipulation. In our study, the pronounced increase in both 2,3-dinor-TXB_2 and 2,3-dinor-6-oxo-

$PGF_{1\alpha}$ immediately after operation may likewise reflect intravascular manipulation. The increase in 2,3-dinor-TXB_2 after operation might be regarded as an exaggeration of the preoperative state, as cardiopulmonary bypass is known to activate platelets and damage the pulmonary endothelium, which is already injured in pulmonary hypertensive children before bypass.[1 2 11 27] The circulating concentration of another vasoactive substance, endothelin, also increases immediately after cardiopulmonary bypass although unlike TXA_2, the circulating concentration of this mediator is normal before operation.[28] Cardiopulmonary bypass damages all endothelia, and the proportion of TXA_2 and endothelin produced by the systemic and pulmonary vascular beds is unknown. Irrespective of source, both TXA_2 and endothelin might be expected to have a greater effect on the more reactive damaged pulmonary vascular bed of these children.[1 2] The immediate postoperative increase in PGI_2, a vasodilator and antiaggregatory substance, might be expected to cause an increase in blood loss.[14] Prostacyclin does not inhibit platelet adhesion to damaged blood vessels at concentrations that prevent platelet aggregation and its therapeutic use has been free of bleeding complications, although not systemic hypotension.[29–31] The function of PGI_2 released locally at sites of incision may therefore be to prevent the thrombus extending from the sites of injury and haemostasis. Its systemic action may help to oppose the excessive vasoconstriction and the hypercoagulable state associated with operation and trauma.

It has been suggested that because of the opposing effects of TXA_2 and PGI_2 on blood vessels and platelets, the ratio of biosynthesis of TXA_2 to PGI_2 may have greater physiological relevance than changes in the biosynthesis of either eicosanoid alone.[8 20] The ratio is abnormal in several different vascular diseases.[6 20 23 32 33] In our study, the ratio of excretion of 2,3-dinor-TXB_2: 2,3-dinor-6-oxo-$PGF_{1\alpha}$ was similar to the control group between 12 and 24 hours after cardiopulmonary bypass on account of the increase in PGI_2. One exceptional case had pulmonary hypertensive crises and in this child the eicosanoid metabolite excretion ratio was above the upper 95% CI of the other children. These findings raise the possibility that the unopposed action of TXA_2 may contribute to the pathogenesis of pulmonary hypertensive crises, and might help to explain why an infusion of PGI_2 is generally more helpful in treating crises than other intravenous vasodilators.[34] Thromboxane receptor antagonism prevents pulmonary hypertension and improves right ventricular function after complement induced pulmonary hypertension in dogs and may prove to be a useful adjunct in the treatment of pulmonary hypertension after cardiopulmonary bypass in humans.[35] Other vasoactive mediators, such as endothelin, may contribute to postoperative pulmonary vascular reactivity, and nitric oxide release may be impaired.[5 15] Nitric oxide has been shown to be an effective pulmonary vasodilator after surgery for congenital heart disease in children.[15] Nevertheless, in our study the rise in the 2,3-dinor-TXB_2: 2,3-dinor-6-oxo-$PGF_{1\alpha}$ excretion ratio during a hypertensive crisis suggests an aetiological role for this vasoconstrictor in pulmonary hypertensive crises.

By the time the patients were discharged from hospital, at a median of 12 days after operation, the excretion rate of 2,3-dinor-TXB_2 was still high. Whether this indicates persistent platelet dysfunction after cardiopulmonary bypass or chronic endothelial injury is unknown. The excretion rate of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ in those who had undergone a successful repair was greater than in the control group. The mechanism of this change is not understood, although surgery is known to stimulate PGI_2 production.[14] Excretion of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ was low, however, after operation in the two patients with a large residual ventricular septal defect. Paradoxically, in vitro an increase in shear stress increases the release of PGI_2.[36] Also, for those patients with a low rate of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ excretion, it was not possible in our study to discriminate between the direct effect of the increased pulmonary blood flow after operation and its probable consequences, left atrial hypertension, and cardiac dysfunction after bypass.

The postoperative increase in excretion of 2,3-dinor-6-oxo-$PGF_{1\alpha}$ after a successful repair meant that when the patients were discharged from hospital the ratio of excretion of the metabolites was similar to that in the control group. A previous study has shown that one year after a successful repair the excretion rates of TXB_2 and $PGF_{1\alpha}$ are normal, indicating recovery of endothelial function.[4] Endothelial dependent relaxation in response to acetylcholine is preserved in young children with an increase in pulmonary blood flow, but is impaired in older patients with fixed pulmonary vascular disease.[15 37] Progressive endothelial dysfunction may be evident as an imbalance in eicosanoid biosynthesis that might contribute to the pathogenesis of pulmonary vascular disease. Although the numbers in our study are small, the return to normal of the ratio soon after intracardiac repair indicates rapid restoration of the balance between vasoconstrictor and dilator mediators and augurs well for the reversibility of the structural abnormalities.

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